REMARKS

Claims 1-85 are pending in the application. Claims 2, 17 and 29-85 are withdrawn from consideration.

Rejection of Claims 1, 3-16, 18, 19 and 22-28 under 35 U.S.C. §103(a)

Claims 11, 3-16, 18, 19 and 22-28 are rejected under 35 U.S.C. §103(a) as being unpatentable over O'Hagan et al., WO 98/33487 (O'Hagan) in view of Hawkins et al., US 6,290,973 (Hawkins). This rejection and its supporting remarks are respectfully traversed.

As noted in the response to the prior Office Action, A proper rejection under 35 U.S.C. 103 requires, *inter alia*, an explanation as to why one of ordinary skill in the art at the time the invention was made would have been motivated to make a proposed modification to the prior art to arrive at the claimed subject matter. See MPEP 706.02(j). See also Examination Guidelines for Determining Obviousness Under 35 U.S.C. 103 in View of the Supreme Court Decision in *KSR International Co.* v. *Teleflex Inc.*, 127 S. Ct. 1727, (2007):

> The Supreme Court in *KSR* noted that the analysis supporting a rejection under 35 U.S.C. 103 should be made explicit. The Court quoting In re Kahn ... stated that " '[R]ejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.' "

The Examiner has asserted that it would have been obvious to a person of ordinary skill in the art at the time the invention was made to combine the teachings of O'Hagan with Hawkins to make an immunogenic composition comprising water, polymer microparticle, antigen adsorbed to microparticle, and various synthetic phospholipids "for the purpose of immunizing a subject to increase or enhance immunogenic activity, immune response or stimulate/enhance protection against an infectious antigen for example."

Rather than providing "some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness" as required by *KSR*, the Examiner has instead taken multiple references disclosing various elements of the claimed invention and combined them together as an obviousness rejection.

Moreover, overlooked in the Examiner's analysis is the fact that there must be a reasonable expectation of success. See MPEP 2143.02 and the cases cited therein. Where immunological adjuvants are concerned, however, one of ordinary skill in the art would not have

a reasonable expectation of success. In this regard, see, for example, the attached article from R. Edelman, *Molecular Biotechnology*, 21(2) 2002, pp.129-148 (Edelman), which demonstrates that those of ordinary skill in the art would have recognized that (a) every adjuvant (including microparticle adjuvants) has a complex and often multi-factorial immunological mechanism, usually poorly understood in vivo, (b) many determinants of adjuvanticity exist and (c) each adjuvanted vaccine is unique. Accordingly, the choice of an adjuvant frequently depends upon experimental trial and error. *Id.*

In view of the foregoing, it is respectfully submitted that, without undue hindsight gained upon review of the present specification and claims, the presently pending claims are unobvious in view of the teachings of Levy and Weiner. See, e.g., MPEP 2142, second paragraph, *Akzo N.V. v. U.S. International Trade Commission*, 808 F.2d 1241, 1480-81, 1 U.S.P.Q.2d, 1241, 1246 (Fed. Cir. 1986), *cert. denied*, 482 U.S. 909 (1987), and *Loctite Corp. v. Ultraseal Ltd.*, 781 F.2d 861, 874, 228 U.S.P.Q. 90-99 (Fed. Cir. 1985).

Nor would not be a reasonable expectation of success.

Consequently, a *prima facie* case of obviousness has not been established by the Examiner. For at least these reasons, reconsideration and withdrawal of the Examiner's rejection are requested.

Rejection of Claims 20 and 21 under 35 U.S.C. §103(a)

Claims 20 and 21 are rejected under 35 U.S.C. §103(a) as being unpatentable over O'Hagan and Hawkins in view of Muttilainen et al., *Microbial Pathogenesis*, 1995, 18:423-436 (Muttilainen) and Cox et al., *Vaccine*, 1997, 15/3:248-256 (Cox). This rejection and its supporting remarks are respectfully traversed.

In particular, it is alleged in the Office Action that it would have been obvious to one of ordinary skill in the art at the time of the invention to combine the teachings of O'Hagan, Hawkins, Muttilainen and Cox with a reasonable expectation of success to prepare the immunogenic compositions as claimed.

In support of the foregoing, the Office Action urges that O'Hagan and Hawkins teach the claimed invention except for the specific antigen, *Neisseria meningitidis* (claim 21) and meningitis B (claim 20).

The deficiencies in O'Hagan and Hawkins are discussed above (including the lack of articulated reasoning with some rational underpinning to support a conclusion of obviousness and the lack of a reasonable expectation of success).

The Examiner further urges (a) that Muttilainen teaches a composition comprising meningitis serogroup B P1 antigen in phospholipid vesicles or liposomes and that the liposome formulation is good as an adjuvant and (b) that Cox teaches that using a combination of adjuvants is desirable to achieve a mix of immunological responses.

Finally, the Examiner urges (a) that *KSR* discloses that if a technique has been used to improve one method, and a person of ordinary skill would recognize that it would be used in similar methods in the same way, then using the technique is obvious unless its application is beyond that person's skill and (b) that the combination of familiar elements according to known methods (e.g., a combination of known adjuvants) is likely to be obvious when it does no more than yield predictable results.

Regarding point (a) in the preceding paragraph, it is noted that the invention presently under examination is an immunogenic composition, as opposed to a method. Concerning point (b), *KSR* involved addressing a known problem with "a finite number of identified, predictable solutions" *KSR*, 127 S. Ct. at 1742. Here, the art is unpredictable (see, e.g., Edelman, which demonstrates that adjuvant selection and combination is a complex and poorly understood endeavor, with the ultimate choice of an adjuvant generally depending upon expensive experimental trial and error). Moreover, there are a near-infinite number of solutions available to the ordinarily skilled artisan (see, e.g., Cox, which evidences a large number of adjuvants, even as of 1997). See also *Ortho-McNeil v. Mylan Laboratories*, 520 F.3d 1358 (Fed. Cir. 2008) ("In sum, this clearly is not the easily traversed, small and finite number of alternatives that KSR suggested might support an inference of obviousness.").

The Examiner's reliance on *KSR* is unfounded as the invention at issue in KSR was in a very predictable art and there was a known problem that was being solved.

Moreover, rather than providing "some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness" as required by *KSR*, the Examiner has instead, with the benefit of undue hindsight, taken random references disclosing various elements of the claimed invention and combined them together as an obviousness rejection.

The Examiner has further created reasons for the claimed combination (i.e., that Cox teaches that using a combination of adjuvants is desirable to achieve a mix of immunological responses) that are simply not supported by adjuvant science. First, as indicated above, adjuvant selection and combination is a complex and poorly understood undertaking.

Moreover, in various instances, a combination of adjuvants is not dictated. One example of this is the *N. meningitidis* serogroup B vaccine from Novartis Vaccine and Diagnostics Inc., which is comprised five proteins with an alum adjuvant. Thus, contrary to the Examiner's assertion that one of ordinary skill in the art would be motivated to combine multiple adjuvants together, this real world example demonstrates that there is no problem to be solved at all, because alum is by itself sufficient.

Finally, the Examiner has ignored the requirement that one of ordinary skill in the art must have a reasonable expectation of success. Such an expectation is unfound here, for example, due to the complex and poorly understood nature of adjuvant action.

For at least these reason, it is respectfully submitted that claims 20 and 21 are patentable over O'Hagan, Hawkins, Muttilainen and Cox.

CONCLUSION

Applicant submits that all pending claims are in condition for allowance, early notification of which is earnestly solicited. Should the Examiner be of the view that an interview would expedite consideration of this Amendment or of the application at large, the Examiner is requested to telephone the Applicant's attorney at (703) 433-0510 in order to resolve any outstanding issues in this case.

FEES

The Office is authorized to charge the two-month extension fee as well as any other fees required to deposit account number 50-1047.

Serial No.: 10/748,117

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I hereby certify that this correspondence is being deposited with the United States Patent and Trademark Office on 12/23/2008 via facsimile to: 571-273-8300.

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(Signature)

REVIEW

The Development and Use of Vaccine Adjuvants

*Robert Edelman**

Abstract

Interest in vaccine adjuvants is intense and growing, because many of the new subunit vaccine candidates lack sufficient immunogenicity to be clinically useful. In this review, I have emphasized modern vaccine adjuvants injected parenterally, or administered orally, intranasally, or transcutaneously with licensed or experimental vaccines in humans. Every adjuvant has a complex and often multi-factorial immunological mechanism, usually poorly understood in vivo. Many determinants of adjuvanticity exist, and each adjuvanted vaccine is unique. Adjuvant safety is critical and can enhance, retard, or stop development of an adjuvanted vaccine. The choice of an adjuvant often depends upon expensive experimental trial and error, upon cost, and upon commercial availability. Extensive regulatory and administrative support is required to conduct clinical trials of adjuvanted vaccines. Finally, comparative adjuvant trials where one antigen is formulated with different adjuvants and administered by a common protocol to animals and humans can accelerate vaccine development.

Index Entries: Vaccine adjuvants; Phase I and II clinical trials; adjuvant mechanisms; adjuvant safety.

1. Introduction

Adjuvants have been used to augment the immune response to antigens for more than 70 years. Ramon first demonstrated that it was possible to increase levels of diphtheria or tetanus antitoxin by the addition of bread crumbs, agar, tapioca, starch oil, lecithin, or saponin to the vaccines (1). In this review, I will provide an overview of how modern vaccine adjuvants are developed and used. First, a general discussion of adjuvants will include definitions of commonly used terms, mechanisms of action, safety, characteristics of an ideal adjuvant, impediments to development, and preclinical and clinical regulatory issues. Finally, I will provide examples of experimental adjuvants that have entered clinical trial to enhance a variety of licensed and experimental vaccines in humans. For additional expositions on this complex subject and for a historical perspective, the reader is referred to recent textbooks on vaccine adjuvants (2–4) and a selection of useful review articles published over the past 21 years (5–14).

Interest in vaccine adjuvants is growing rapidly for several reasons. First, dozens of new vaccine candidates have emerged over the past decade to prevent or treat infectious diseases, cancer, fertility, allergic, and autoimmune diseases. Many of these candidates require adjuvants. Second, vaccines have become commercially more profitable in the past few years. Third, the Children's Vaccine Initiative (CVI) initiated in 1990 (15), and the Global Alliance for Vaccines initiated in 1999 (16), have helped to energize political and public health interest in vaccine adjuvants by establishing ambitious goals for enhancing present vaccines and for developing new ones. Fourth, refinements in the fields of analytical biochemistry, macromolecular purification, recombinant technology, and improved understanding of immunological mechanisms and disease pathogenesis have helped to improve the technical basis for adjuvant development and application. Finally, the development of experimental adjuvants has been driven by the failure of aluminum

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compounds to enhance many vaccines in humans, to enhance many subunit vaccine antigens in animals, or to stimulate cytotoxic T-cell responses.

2. Definitions

The discussion of vaccine adjuvants will be facilitated by a definition of terms.

2.1. Adjuvant

The term "adjuvant" (from the latin, adjuvare = help) was first coined by Ramon in 1926 for a substance used in combination with a specific antigen that produces more immunity than the antigen used alone (17). The enormous diversity of compounds that increase specific immune responses to an antigen and thus function as vaccine adjuvants makes any classification system somewhat arbitrary. Adjuvants in Table 1 are grouped according to origin rather than according to mechanism of action, because the mechanism for most adjuvants are incompletely understood. By contrast, Cox and Coulter (11) have classified adjuvants into two broad groups, particulate or non-particulate. A third classification scheme, modified from Audibert and Lise (18), identifies at least four main sources of adjuvants, as follows: (1) botanical, e.g, saponin or glucan extract; (2) bacterial, e.g., muramyl dipeptides, monophosphoryl lipid A, cholera toxin, and CpG oligodeoxynucleotides; (3) chemical, e.g., aluminum salts, pluronic block polymers, lactide and glycolide, and polyphosphazenes; (4) cytokines and hormones, e.g., interleukin-2, granulocyte-macrophage colony stimulating factor, and dehydroepiandrosterone.

2.2. Carriers, Vehicles, and Adjuvant Formulations

Several terms used in **Table 1** need to be defined. A "carrier" has several meanings. It is an immunogenic protein bound to a hapten or a weakly immunogenic antigen (19). Carriers increase the immune response by providing T cell help to the hapten or antigen (20,21). Alternatively, a carrier may also be a living organism (or vector) bearing genes for expression of the foreign hapten or antigen (22–27). A DNA vaccine is a carrier in the sense that, like some living vectors, it carries a plasmid-based DNA vector encoding the production of the protein antigen upon inoculation into the host (28,29).

A "vehicle" provides a substrate for the adjuvant, the antigen, or the antigen–carrier complex. Unlike carriers, vehicles are not immunogenic. Some vehicles provide a fairly consistent adjuvant effect (30,31), while others do not (32). The immunostimulatory effects of vehicles are often augmented by the addition of conventional adjuvants to constitute "adjuvant formulations," as discussed below.

Examples of adjuvant formulations tested in humans with a variety of antigens (and with variable success) include: monophosphoryl lipid A and cell wall skeleton of Mycobacterium phlei adjuvants in a squalane-in-water emulsion vehicle (33), monophosphoryl lipid A adjuvant in a liposome vehicle (34), threonyl-muramyl dipeptide adjuvant and Pluronic L-121 block polymer adjuvant in a vehicle emulsion of squalane and Tween 80 (35), muramyl tripeptide-dipalmitoyl phosphatidylethanolamine adjuvant in a squalene-in-water emulsion vehicle (36), and monophosphoryl lipid A and QS-21 adjuvants in a proprietary oil-in-water emulsion (37).

3. Examples of Modern Vaccine Adjuvants Used in Animals and Humans

3.1. Adjuvants for Parenteral Vaccines

Agents listed in **Table 1** are examples of the many varieties of immunopotentiators used during the past 30 years. The majority have been injected intramuscularly or parenterally. The majority are being developed and tested by industry. The list of adjuvants is incomplete, because I have not conducted an exhaustive literature search, because the results have appeared in abstracts in non-indexed publications, and because many studies are proprietary. The adjuvants marked by an asterisk in Table 1 have completed trial in humans, or they are now undergoing clinical trial. Promising adjuvants not yet tested in humans are also listed. In some instances, adju-

Table 1
Classes of Modern Vaccine Adjuvants

1. Mineral Salts	2. Surface-active agents and Microparticles	3. Bacterial Products	4. Cytokines and Hormones	5. Unique Antigen Constructs
Aluminum ("Alum") Aluminum hydroxide * Aluminum phosphate * Calcium phosphate *	Nonionic block polymer surfactants * Virosomes * Ty-virus-like particles * Saponin (QS-21) * Meningococcal outer membrane proteins (Proteosomes) * Immune stimulating complexes (ISCOMs) * Cochleates Dimethyl dioctadecyl ammonium bromide (DDA) Avridine (CP20,961) Vitamin A Vitamin E	Cell wall skeleton of *Mycobacterium phlei* (Detox^{R}) * Muramyl dipeptides and tripeptides Threonyl MDP (SAF-1) * Butyl-ester MDP (Murabutide^{R}) * Dipalmitoyl phosphatidylethanolamine MTP * Monophosphoryl lipid A * *Klebsiella* pneumonia glycoprotein * *Bordetella pertussis* * Bacillus Calmette-Guérin * *V. cholerae* and *E. coli* heat labile enterotoxin * CpG oligodeoxynucleotides * Trehalose dimycolate	Interleukin-2 * Interleukin-12 * Interferon-alpha * Interferon-gamma * Granulocyte-macrophage colony stimulating factor * Dehydroepiandrosterone * Flt3 ligand * 1,25-dihydroxy vitamin D_3 Interleukin-1 Interleukin-6 Human growth hormone 2-microglobulin Lymphotactin	Multiple peptide antigens attached to lysine or polyoxime core (MAP) * CTL epitope linked to universal helper T cell epitope and palmitoylated at the N terminus (Theradigm-HBV) *

6. Polyanions	7. Polyacrylics	8. Miscellaneous	9. Carriers	10. Living Vectors	11. Vehicles
Dextran Double-stranded polynucleotides	Polymethyl-methacrylate Acrylic acid cross-linked with allyl sucrose (Carbopol 934P)	N-acetyl-glucosamine-3yl-acetyl-L-alanyl-D-isoglutamine (CGP-11637) * Gamma inulin + aluminum hydroxide (Algammulin) * Transgenic plants * Human dendritic cells * Lysophosphatidyl glycerol Stearyl tyrosine Tripalmitoyl pentapeptide	Tetanus toxoid * Diphtheria toxoid * Meningococcal B outer membrane protein (proteosomes) * *Pseudomonas* exotoxin A * Cholera toxin B subunit * Mutant heat labile enterotoxin of enterotoxigenic *E. coli* * Hepatitis B virus core * CpG dinucleotides * Cholera toxin A fusion proteins Heat shock proteins Fatty acids	Vaccinia virus * Canarypox virus * Adenovirus * Yellow fever vaccine virus * Attenuated *Salmonella typhi* * Attenuated *Shigella* * Bacillus Calmette-Guérin * *Streptococcus gordonni* * Herpes simplex virus Polio vaccine virus rhinovirus Venezuelan equine encephalitis virus Sindbis virus *Yersinia enterocolitica* *Listeria monocytogenes* *Bordetella pertussis* *Saccharomyces cerevisiae*	Water-in-oil emulsions Mineral oil (Freund's incomplete) * Vegetable oil (peanut oil) * Squalene and squalane * Oil-in-water emulsions Squalene + Tween 80 + Span 85 (MF59) * Liposomes * Biodegradable polymer microspheres Lactide and glycolide * Polyphosphazenes * Beta-glucan Proteinoids

* Identifies adjuvants administered to humans. Of these, only aluminum salts, virosomes, and MF59 are adjuvants approved as licensed vaccine formulations in the United States

vants have been combined in an adjuvant formulation hoping to gain a synergistic or additive effect.

3.2. Vaccine Adjuvants versus Non-specific Enhancers of Immunity

Agents listed in **Table 1** enhance specific antigens and are administered concurrently with the antigen. Adjuvants not administered in a single dose at or near the time of antigen inoculation and into the same injection site as the antigen, are not listed. Thus, adjuvants administered repeatedly as non-specific enhancers of immune response are largely excluded. Immunopotentiating agents administered to humans separately in time or location from the vaccine may be impractical for vaccinating large numbers of persons, and are potentially unsafe because of their physiological effects on the entire body. They may have a role, however, in immunizing a small number of high risk, immuno-incompetent individuals, such as renal dialysis patients at risk for hepatitis B or the very elderly at risk of influenza. Examples of such "whole body" adjuvants used in humans to augment vaccines include Na diethyldithiocarbamate (38), thymosin alpha one (39), loxoribine (40), granulocyte-macrophage stimulating factor (41), cimetidine (42), and dehydroepiandrosterone sulfate (43). The results of such trials to date have been disappointing.

3.3. Adjuvants for Mucosal Vaccines

Recent advances in vaccinology have created an array of vaccines that can be delivered to mucosal surfaces of the respiratory, gastrointestinal, and genitourinary tracts using intranasal, oral, and vaginal routes (44). The development of mucosal vaccines has come at a time when the use of the syringe and needle for parenteral vaccination is losing favor. There are several reasons for this. First, the contamination of reused needles and syringes with HIV, hepatitis B, and hepatitis C viruses is a growing hazard, particularly in developing countries of Africa and Asia. Second, the number of marketed, parenteral pediatric vaccines are increasing worldwide. Currently, 20 separate vaccine injections are administered to U.S. infants over the first 18 months of life. Parents and physicians are demanding fewer injections. Third, vaccines administered mucosally, compared to vaccines administered parenterally, may provide better protection against the numerous respiratory, gastrointestinal, and genital pathogens that infect and proliferate at mucosal surfaces. Well-tolerated adjuvants that enhance such vaccines will play an important role in mucosal immunization. Some of the more promising adjuvants completed or near clinical trial include microspheres composed of copolymers of lactic and glycolic acids (45,46); proteosomes (47,48), liposomes (49), CpG DNA (50), cochleates (51), and virus-like particles (52).

Cholera toxin (CT) and the closely related, heat-labile enterotoxin of enterotoxigenic *Escherichia coli* (LT) are powerful adjuvants that augment the local and systemic serum antibody response to coadministered antigens, particularly when delivered by the mucosal route (53–60). Mutant CT and LT molecules have been engineered to reduce toxicity but to retain sufficient adjuvanticity to enhance local IgA, systemic IgG, and cellular immune responses to co-administered vaccine antigens (61–64). Clinical trials using mutant LT toxins as adjuvants of nonliving vaccine antigens are in progress (13). Recent safety concerns, engendered by passage of CT and LT into the olfactory bulb of Balb/C mice after intranasal instillation, must be resolved before clinical evaluation of these powerful adjuvants as intranasal adjuvants can proceed (65).

Attenuated recombinant bacteria (26,66,67) and viruses (22), administered orally as live vectors of cloned genes encoding protective antigens of other pathogens, have undergone phase I trials to stimulate immune effector responses. Most of these early attempts to stimulate mucosal immune responses in volunteers using live vectors have only been marginally successful. The first attempts to immunize volunteers against LT and Norwalk virus antigen encoded in a transgenic potato and administered as edible vaccines were more successful (68,69). It remains to be seen if

other protein antigens (e.g., HBsAg) when given via transgenic plants will be immunogenic or will instead induce tolerance to the antigen.

3.4. Adjuvants for Transcutaneous Vaccines

Another needle-free method of immunization is via the transcutaneous route (70—72). The skin is a robust immunological organ heavily populated with Langerhans' antigen-presenting cells (73). Transcutaneous vaccination involves topical application of antigens and a variety of adjuvants to intact skin using a simple occlusive patch (72). The ability of skin to process foreign antigens has been exploited by other vaccine strategies, e.g., immunization with the "gene gun," which injects plasmid DNA through the stratum corneum (74), smallpox vaccine via scarification, and BCG vaccine via intradermal injection (27). By utilizing the proper adjuvant in mice, it is possible to induce both systemic and mucosal immunity via the skin (75, 76). In humans, CT applied to the skin with tetanus toxoid induced systemic immunity to the toxin (70), and a systemic immune response was engendered when LT was co-administered with a pilus protein of E. coli as a prototype traveler's diarrhea vaccine (77). Mucosal responses were not measured. This novel approach to vaccine delivery, if found to be safe and non-reactogenic in continuing studies, will be aggressively developed for a variety of preventive and therapeutic vaccines.

4. Mechanisms of Adjuvant Action

To date, most subunit vaccines are poor antigens, be they natural products, recombinant products, or synthetic peptides. Subunit antigens fail for a variety of reasons, such as incorrect processing by the immune system, rapid clearance, stimulation of inappropriate immune response, and lack of critical B-cell or T-cell epitopes. Potentially, some of these failures can be overcome by administering subunit antigens with adjuvants. It should be remembered, however, that the best adjuvant will never correct the choice of the wrong (non-protective) epitope.

Traditional live vaccines or whole-cell inactivated microbial vaccines are generally better immunogens than subunit vaccines. Live and inactivated whole organisms are structurally more complex than subunit vaccines, and so contain many redundant epitopes, which offer more opportunity to bypass genetic restriction of the vaccinee. Such vaccines also provide a larger antigen mass than subunit vaccines, particularly if they replicate in vivo. Their antigens are larger molecules, portions of which may serve as carrier proteins and thus function as intrinsic adjuvants to enhance immunogenicity by providing T cell help. Finally, bacterial DNA may directly stimulate the host's immune system due to it large content of unmethylated CpG dinucleotides (78), and whole bacterial vaccines may contain CpG DNA.

4.1. Specific Immune Mechanisms

Some mechanisms of adjuvant action are discussed below and are summarized in **Table 2**. Vaccine adjuvants can (1) increase the potency of small, antigenically weak synthetic or recombinant peptides. (2) They can enhance the speed, vigor, and persistence of the immune response to stronger antigens. For example, aluminum adjuvants used with licensed pediatric vaccines (e.g., DTP) elicit early and higher antibody response after primary immunization than do unadjuvanted preparations. (3) Adjuvants can increase the immune response to vaccines in immunologically immature, immunosuppressed, or senescent individuals. (4) Adjuvants can select for or modulate humeral or cell-mediated immunity, and they can do this in several ways. First, antigen processing can be modulated, leading to vaccines which can elicit both helper T cells and cytotoxic lymphocytes (CTL) (reviewed in 8, 79). Second, depending upon the adjuvant, the immune response can be modulated in favor of MHC class I or MHC class II response (8, 79). For example, the QS-21 adjuvant can elicit MHC class I CTL responses when mixed with protein antigens, peptides, or inactivated viruses (80, 81). Aluminum adjuvants, among others, elicit principally MHC class II antibody responses when combined with protein antigens or inactivated organisms (79, 82). Third, adjuvants can modulate the immune response by preferentially stimulating Th1 or Th2

Table 2
Some Mechanisms of Adjuvant Action

- Stabilizes epitope conformation
- Generates a depot at the site of inoculation with slow release of antigen
- Targets the antigen to antigen-presenting cells (APCs) by formation of multimolecular aggregates, or by binding antigen to a cell-surface receptor on APCs.
- Directs antigen presentation by major histocompatibility complex (MHC) class I or MHC class II pathways, by means of fusion or disruption of cell membranes, or by direct peptide exchange on surface MHC molecules.
- Preferentially stimulates Th1 or Th2 CD4+ T-helper cells or CD8+ cytotoxic T-lymphocytes, by modulation of the cytokine network in the local microenvironment.

Table 3
Beneficial Effects of Vaccine Adjuvants

- Increase the potency of antigenically weak peptides
- Enhance the speed, vigor, and persistence of the immune response to stronger antigens
- Modulate antibody avidity, specificity, quantity, isotype, and subclass
- Select for or enhance the cytotoxic T cell response
- Increase the immune response to vaccines in immunologically immature, suppressed, or senescent individuals
- Decrease the amount of antigen required, thus reducing the cost and the likelihood of antigen competition in combination vaccines

CD4+ T-helper cells (83). The Th1 response is accompanied by secretion of interleukin-2 (IL-2), interferon-gamma (IFN-γ), and TNF-beta leading to a CMI response, including activation of macrophages and CTL and high levels of IgG2a antibodies in mice. The Th2 response is modulated by secretion of IL-4, IL-5, IL-6, and IL-10, which provide better help for B cell responses, including those of IgG1, IgE and IgA isotypes in mice. Aluminum salts principally stimulate the Th2 response (84), while the Th1 response is stimulated by manyadjuvants, such as muramyl dipeptide, monophosphoryl lipid A, and QS-21 (8,35,85,86). (5) Vaccine adjuvants can modulate antibody avidity, specificity, quantity, isotype, and subclass against epitopes on complex immunogens (9,87,88). For example, only certain adjuvants, vehicles, and adjuvant formulations can induce the development of the protective IgG2a antibody isotype against *Plasmodium yoelii* (9). (6) Vaccine adjuvants can decrease the amount of antigens in combination vaccines, thus reducing the likelihood of antigen competition and carrier-specific epitope suppression. In addition, by reducing the quantity of antigen needed to protect, adjuvants can decrease the cost and increase the availability of vaccines. On the other hand, the high cost of some modern adjuvants may offset the savings realized by the reduced antigen requirement, thereby paradoxically driving up vaccine cost overall.

One must remember that in vivo, most adjuvants have complex and multifactorial immunological mechanisms, often poorly understood. The immunological mechanisms utilized by many adjuvants are under investigation. Such investigations will provide answers to some of the following questions. Does the adjuvant induce cell mediated (Th1) immunity, humoral (Th2) immunity, or a balance of Th1 and Th2? Which IG isotypes dominate? Which cytokines are induced? Are CD4+ T-helper cells or CD8+ cytotoxic T-lymphocytes induced? The list of such questions is extensive, and grows in proportion to our understanding of immunological mechanisms in general.

5. Advantages of Adjuvants

Vaccine adjuvants influence the immune response to our benefit in one or more ways (**Table 3**). The ability of adjuvants to influence so

Table 4
Modulators of Vaccine Adjuvant Effects

- Route
- Timing
- Dose
- Adjuvant formulation
- Antigen construct
- Host dpecies
- Intra-species genetic variation
- Immune status of the host

many parameters of the immune response greatly complicates the process of finding an effective adjuvant. This is because our knowledge of how any one adjuvant operates on a cellular level is insufficient to support a completely rational approach for matching the vaccine antigen with the proper adjuvant. Consequently, many investigators advocate an empirical approach for antigen selection based on the balance among toxicity, adjuvanticity in animals, and whether one wishes to stimulate a Th1 response, a Th2 response, or a balance of the two responses. Finally and importantly, one must remember that the advantages of adjuvants are modulated strongly by the immunization schedule and route, by the antigen and adjuvant formulation, and by the host (**Table 4**).

6. Safety

The most important attribute of any adjuvanted vaccine is that it is more efficacious than the aqueous vaccine, and that this benefit outweighs its risk. During the past 70 years many adjuvants have been developed, but they were never accepted for routine vaccination because of their immediate toxicity and fear of delayed side effects. The current attitude regarding risk-benefits of vaccination in our Western society favors safety over efficacy when a vaccine is given to a healthy population of children and adults. In high risk groups, including patients with cancer and AIDS, and for therapeutic vaccines, an additional level of toxicity may be acceptable if the benefit of the vaccine was substantial.

Unfortunately, the absolute safety of adjuvanted vaccines, or any vaccine, cannot be guaranteed,

Table 5
Real and Theoretical Risks of Vaccine Adjuvants

1. Local acute or chronic inflammation with formation of painful abscess, persistent nodules, ulcers, or draining lymphadenopathy
2. Influenza-like illness with fever.
3. IgE-type immediate hypersensitivity to vaccine antigen, including anaphylaxis.
4. Chemical toxicity to tissues or organs.
5. Induction of hypersensitivity to host tissue, producing autoimmune arthritis, amyloidosis, anterior uveitis.
6. Cross-reactions with human tissue antigens, causing glomerulonephritis or meningoencephalitis.
7. Immune suppression or oral tolerance
8. Carcinogenesis
9. Teratogenesis or abortogenesis
10. Spread of a live vectored vaccine to the environment

so we must minimize the risks. The concern about adjuvant safety has encouraged continued use of aluminum adjuvants because of their long record of relative safety in children. Safety concerns have helped justify the development of unique synthetic antigen constructs and DNA vaccines not dependent on adjuvants. For example, large polymerized monomers of haptens and peptides have been linked together in a multimeric form designed to increase intrinsic adjuvanticity (multiple antigen peptide systems [MAPs]) (89,90). The first phase 1 trials of DNA-based vaccines showed them to be safe (28,29,74). It remains to be seen if MAPs, DNA vaccines, and other unique antigen constructs will retain enough inherent adjuvanticity to avoid the risk of administering them with extraneous chemical or biological adjuvants to humans. The fact remains that, in general, the inflammatory reaction induced by most adjuvants seems to enhance adjuvanticity, so the more robust the adjuvanticity, the more robust the reactogenicity to that adjuvant. The clinical goal is to arrive at an acceptable balance between adjuvanticiy and reactogenicity.

The real or theoretical risks of administering vaccine adjuvants have been discussed in detail (5,6,91,92) and are summarized in **Table 5**.

Undesirable reactions can be grouped as either local or systemic.

6.1. Local Reactions

The most frequent adverse side effect associated with adjuvanted vaccines is the formation of local inflammation with signs of swelling and erythema, and symptoms of tenderness to touch and pain on movement. Such reactions occur more frequently in preimmune individuals, or after repeated immunization (33,93,94). The inflammation is thought to be the result of formation of inflammatory immune complexes at the inoculation site by combination of the vaccine antigen with preexisting antibodies and complement, resulting in an Arthus-type reaction. Such reactions tend to occur more frequently after adjuvanted vaccines than after aqueous vaccines because of the high antibody titers induced by adjuvants. In addition, inflammatory cytokines released by many adjuvants contribute to both the local inflammation and systemic flu-like symptoms.

Painful abscesses and nodules at the inoculum site are seen, but far less frequently (reviewed in 5). Possible mechanisms for such local reactions include: (1) contamination of the vaccine at the time of formulation with reactogenic chemicals and microbial products; (2) instability of the vaccine on storage with breakdown into reactogenic side products; and (3) poor biodegradability of the adjuvanted vaccine resulting in prolonged persistence in the tissues and reactive granuloma formation. Such local reactions are of special concern for depot-type adjuvants, such as aluminum salts, liposomes, biodegradable polymer microspheres, and, especially, oil emulsions. Severe local reactions in humans have followed injections of vaccines adjuvanted with IFA (incomplete Freund's adjuvant) (reviewed in 5), DETOX™ (monophosphoryl lipid A + cell wall skeleton of *M. phlei* + squalane oil vehicle + Tween 20 emulsifier) (33,95), muramyl tripeptide covalently linked to dipalmitoyl phosphatidylethanolamine (MTP-PE) in a squalene-in-water emulsion (96), and the squalene oil adjuvant, Montanide ISA 720 (97).

We have noted development of local ulceration for as long as 70 d after intradermal inoculation of volunteers with a recombinant BCG-OspA Lyme disease vaccine; the open sores drained viable rBCG-OspA before they spontaneously healed (27). Development of similar draining sores occur commonly in adults after intradermal inoculation with standard BCG vaccine (98,99). We and others have observed a "recall reaction," characterized by immediate swelling, hives, and intense pruritus at the skin site of a previous antigen injection within 5–20 min after reexposure to that antigen at a remote site (100–102). The reaction seem to be associated with circulating IgE antibody or high-titered serum antibody of yet unknown isotype.

Severe local pain has occurred immediately after intramuscular injection of 15 of 108 volunteers administered a recombinant HIV protein formulated with QS-21 (103). Although the pain lasted from several minutes to several hours and was associated with several vasovagal reactions in several volunteers, no long-lasting side effects were reported. Addition of excipients, such as Triton X-100, to QS-21 formulations has eliminated severe painful injections without affecting adjuvanticity (C.R. Kensil, personal communication).

Finally, 14 of 19 volunteers immunized transdermally with LT and an *E. coli* pilus antigen developed a localized, pruritic, contact-dematitis-like rash at the vaccination site. The rash began 1 to 2 d after the second or third application and lasted 5–7 d. A skin biopsy was compatible with cutaneous delayed type sensitivity (77). Such local reactions may impede the development of transcutaneous immunization.

6.2. Systemic Reactions

Anterior chamber uveitis has been reported with MDP and several MDP analogs in rabbits (104) and monkeys (105). Anterior uveitis has been systematically sought in at least one adjuvant vaccine study involving 110 volunteers, but it was not found (106). A slit lamp examination of volunteers to detect subclinical uveitis is not commonly performed. Adjuvant-associated arthritis (107–109) has not been reported in humans, even after long-term followup (110–113). More theo-

Table 6
Characteristics of the Ideal Adjuvant

1. It must be safe, including freedom from immediate and long-term side effects.
2. It should be biodegradable or easily removed from the body after its adjuvant effect is exhausted to decrease the risk of late adverse effects.
3. It should elicit a more robust protective or therapeutic immune response combined with the antigen than when the antigen is administered alone.
4. It must be defined chemically and biologically, so that there is no lot-to-lot variation in the manufactured product, thereby ensuring consistent responses in vaccinees between studies and over time.
5. Efficacy should be achieved using fewer doses and/or lower concentrations of the antigen.
6. It should be stable on the shelf to be commercially and clinically useful.
7. The adjuvant should be affordable.

retical risks include the induction of autoimmunity or cancer. Fortunately, in 10 and 18 year followup studies, the incidence of cancer, autoimmune, and collagen disorders in 18,000 persons who received oil-emulsion influenza vaccine in the early 1950s was not different from that in persons given aqueous vaccines (30, 112). A 35 yr follow up of these of protocols was 1.5 yr, and the mean number of immunizations was 3.5. The candidate vaccines without adjuvant were generally well tolerated. The only adverse effects clearly related to vaccination were associated with moderate to severe local pain or inflammation, self-limited in nature, that were associated with the adjuvants, particularly alum